Molecular Markers Associated with Resistance to the Root-Knot Nematode *Meloidogyne arenaria* from *Vitis mustangensis*

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Abstract

Damages caused by root-knot nematodes can be counteracted using resistant rootstocks. In order to discover the genes underlying nematode resistance in *Vitis* germplasm, we examined a population segregating for nematode resistance. The population was obtained from the cross between 'Harmony' (susceptible female parent) and a selection of *Vitis mustangensis* (heterozygous resistant male parent). Resistance and susceptibility to *Meloidogyne arenaria* was evaluated by counting the number of egg masses on root systems of individual seedlings six weeks after inoculation. We tested 210 10-mers primers of kits A, B, C, E, G, I, J, M, N, O, P, U, and Y from Operon Technologies. We used the BSA (Bulked Segregant Analysis) method to compare two bulks, constituted by DNA of nine resistant and nine susceptible individuals, respectively. Six different polymorphic RAPD markers were identified in a range between 300 and 3000 bp that are associated with the resistance trait.

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) can cause lesions and modifications (knots and galls) on grapevine roots that reduce water and nutrient uptake, limit growth, and provide entry points for other pests and pathogens. In grape growing regions with light and sandy soils and warm climates, root-knot nematodes can cause serious damage to grapevines. Using resistant rootstocks is an effective tool for grape cultivation on soils infested by root-knot nematodes. The genetics of *Vitis* resistance to root-knot nematodes were described first by Lider (1954), who identified the N allele in *V. mustangensis*, *V. solonis*, and the rootstock 1613 Couderc. The rootstocks 'Harmony' and 'Freedom' derive their N allele nematode resistance from 1613C and Dog Ridge. Root-knot nematode populations have evolved to attack also 'Harmony' and 'Freedom'; these strains are identified as "N virulent" nematodes. Accessions of several *Vitis* species have been identified that are genetic sources of resistance against N virulent nematodes. In this paper, we present the analysis of a segregating population infected with N virulent strains of *Meloidogyne arenaria.*

MATERIALS AND METHODS

The female parent was the rootstock 'Harmony', clonal selection 4 from the University of California, Foundation Plant Services program. The vines used for crosses grew in the rootstock collection of the University of California, Davis, Department of Viticulture and Enology. The male parent was *Vitis mustangensis,* accession number DVIT 1842, from the collection of the United States National Clonal Germplasm Repository, Davis, California. *Vitis mustangensis* is a wild grapevine species native to eastern Texas, Oklahoma, Arkansas, and Louisiana. Cousins et al. (2002) found this accession to be heterozygous for a dominant allele for resistance to the virulent *M. arenaria* nematodes used for screening this population. The crosses were made in the spring of 2000. The seedlings grew in greenhouse at the Grape Genetics Research Unit, US Department of Agriculture, Agricultural Research Service, Geneva (NY), and they were inoculated with nematodes. After screening for nematode reproduction, the seedlings were propagated as vegetative cuttings and grown in pots in the greenhouse.

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Resistance to *M. arenaria* was evaluated using the method of Cousins and Walker (2001), counting egg masses six weeks after inoculation and weighing the roots.

Nine resistant (R) and nine susceptible (S) seedlings were used for DNA analysis (Table 1). DNA was extracted from young leaves following the procedure of Lin and Walker (1997).

A total of 210 oligonucleotide primers (10-mers) from Operon Technologies (Alameda, CA, USA) kits A, B, C, E, G, I, J, M, N, O, P, U, and Y were tested, using BSA (Bulked Segregant Analysis) and comparing the two bulks, constituted respectively by nine resistant individuals and nine susceptible ones.

The reaction mixture included 1X reaction buffer, 2 mM $MgCl_2$, 200 µM of dNTP, 0.35 µM of single 10-base primer, 10 ng of bulked DNA, and 0.5 U of Taq DNA-polymerase (Bioline, London, U.K.), d-H_2O was added to bring the final volume to 20 µl. DNA amplification was carried out in a Perkin-Elmer Thermal Cycler (Mod. GeneAmp PCR System 2400, Foster City, CA, USA) programmed for an initial denaturation of 4 min 40 s at 94°C, followed by 40 cycles of 15 s at 94°C, 30 s at 35°C, 2 min at 72°C, and finally by 7 min extension step at 72°C. Ten µl of each amplified sample were loaded onto a 0.8% agarose gel with 4 µl of ethidium bromide and separated by electophoresis in 1X TAE buffer. A DirectLoad™ Wide Range DNA Marker (Sigma-Aldrich) ranging from 50 to 10,000 bp was used as molecular weight marker. Polymorphic markers found in the BSA were then amplified in the 18 seedlings individually.

RESULTS AND DISCUSSION

Among the 210 primers tested by BSA, six of them (OPI 7, OPI 19, OPJ 13, OPO 15, OPU 9, and OPY 18) showed polymorphisms between the bulks and were selected to confirm their ability to discriminate resistant from susceptible seedlings. The six selected primers, which generated RAPD fragments with size ranging from 300 to 3,000 bp, were used to screen all the 18 seedlings individually for the presence or absence of the polymorphic markers identified in the bulks. We found some bands that showed a skewed distribution between the resistant and the susceptible individuals, but none of them was tightly linked with the trait. Due to the small number of examined individuals and the occurrence of recombination, these markers are not conclusively linked to the resistance character. Our best marker was obtained using the primer OPI 7 (CAGCGACAAG). A resistant band of 1,400 bp was present in seven out nine resistant individuals, and absent in seven out nine susceptible individuals (Fig. 1). As our results were not definitive, we seek to improve analysis of this trait with more primers and a larger segregating population.

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Literature Cited

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Tables

Table 1. Progeny obtained from the cross 'Harmony' x *Vitis mustangensis* with egg-mass counts and root weight of the individuals used in marker development.

Seedling number	Identification	Resistance class	Egg masses	Root weight (g)
1	PC00128-32	resistant	2	4.74
2	PC00128-46	resistant	0	11.11
5	PC00128-47	resistant	0	12.07
6	PC00128-39	resistant	1	8.29
7	PC00128-44	resistant	0	6.56
8	PC00128-38	resistant	0	11.82
18	PC00128-49	resistant	1	12.54
19	PC00128-64	resistant	2	20.74
20	PC00128-50	resistant	2	14.34
3	PC00128-8	susceptible	48	5.03
4	PC00128-6	susceptible	17	7.46
11	PC00128-22	susceptible	51	5.19
12	PC00128-21	susceptible	39	7.16
13	PC00128-28	susceptible	27	11.32
14	PC00128-83	susceptible	71	11.44
15	PC00128-81	susceptible	39	12.41
16	PC00128-26	susceptible	74	3.46
17	PC00128-23	susceptible	189	8.91

Figures

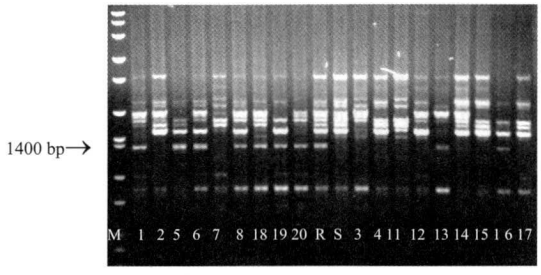

Fig. 1. RAPD profiles obtained with primer OPI7 for grapevines that are resistant (left) and susceptible (right) to *Meloidogyne* nematodes. R and S are results from the bulk of DNAs from the resistant and susceptible individuals. A polymorphic band of about 1400 bp is evident.